Application of Thermoresponsive Polymer and Microfluidics to the Development of a Velocity-Dependent Cell-Sorting Microdevice

Jesse Fine[1], Natalia Higuita-Castro[1, 2], Vasudha Chaurey-Shukla[1], Daniel Gallego-Perez[1,3]

[1]Department of Biomedical Engineering, The Ohio State University, Columbus OH 43210

[2]Division of Pulmonary, Critical Care, and Sleep Medicine; The Ohio State University; Columbus OH 43210

[3]Department of Surgery, The Ohio State University, Columbus OH 43210

Advisory Committee:

Dr. Daniel Gallego-Perez, Adviser

Dr. Derek J Hansford, Co-Adviser

Completed in Partial Fulfillment of the Requirements for Graduation with Research Distinction in the Department of Biomedical Engineering at The Ohio State University

Vita

Education

The Ohio State University est. 2018
B.S. Biomedical Engineering

Lynbrook Senior High School 2014
Advanced Regents Diploma with Distinction

Publications

Yue, T., Jia, X., Petrosino, J., Wang, D., Sun, L., Fan, Z., **Fine, J**., Davis, R., Galster, S. Kuret, J. Scharre, D., Zhang, M. 2017. 'Computational Integration of Nano-Scale Physical Biomarkers and Cognitive Assessments for Alzheimer's Disease Diagnosis and Prognosis.' Science Advances. 3(7)

Abstracts & Presentations

POSTER

1. **Fine, J**., Higuita-Casto N., Chaurey-Shukla V., Gallego-Perez D. 'Application of Thermoresponsive Polymer to the Development of a Velocity-Dependent Cell-Sorting Microdevice'. 2018. Denman Research Forum. Columbus, OH, April 2018
2. **Fine, J**., Lieser, C., Middendorf, M., Galster, S., Zhang, M. 'Extended Kalman Filter-based Computational Integration of Multi-Sensor Data for Human Cognitive Assessment and Performance Prediction'. 2017. Brain Health and Performance Summit. Columbus, OH, April 2017.
3. Sun, L., Fan, Z., Yue, T., **Fine, J**., Lee, G., Davis, R., Kuret, J., Scharre, D., 2. Zhang, M. Lab-on-a-chip 'Self- assembly of Fluorescent Peptide-based Nanoparticles for Blood-based Diagnosis of Alzheimer's Disease'. 2016. Biomedical Engineering Society. Minneapolis, Mn, October 2016
4. Yue, T., Jia, X., Petrosino, J., Wang, D., Sun, L., Fan, Z., **Fine, J**., Davis, R., Galster, S. Kuret, J. Scharre, D., Zhang, M. 'Computational Integration of Nano-Scale Physical Biomarkers and Cognitive Assessments for Alzheimer's Disease Diagnosis and Prognosis'. 2016. Biomedical Engineering Society. Minneapolis, Mn, October 2016

PLATFORM PRESENTATION

1. Noble, G., Bodnyk, K., Litsky, A., **Fine, J**., Pagnotti, G., Rubin, C., Fitzpatrick, N., Allen, M., Hart, R. 'Low Intensity Vibration Improves Endoprosthesis Osseointegration in an Ovine Model'. Biomedical Engineering Society. Tampa, FL, October 2015.

Abstract

Low-cost velocity dependent cell sorting in 2D is a currently nonexistent technology for cancer research. The development of such a device would enable further research on the treatment of various deleterious cancers, such as Glioblastoma Multiforme (GBM), which metastasize based off the high motility of a single cell. Here we present a low-cost device capable of sorting these cells. Separation would enable development of highly specific therapeutic agents to limit cancer metastasis in patients. This device consists of microfluidics channels situated under microtextured Polydimethylsiloxane (PDMS) coated with the thermoresponsive polymer Poly(N-isopropylacrylamide) (PNIPAM). Cells are seeded on one end of the device and orient themselves parallel to the striations patterned into the PDMS; traveling further across the device over time. At a specific location (determined by velocity of target cells and time passed), low-temperature fluid can be passed through the microfluidic channel below which triggers a selective conformational change in the PNIPAM. This change shifts PNIPAM from nonpolar to polar, causing the polymer to release previously-adhered cells into solution in favor of binding to media. Establishing the PNIPAM layer capable of releasing cells while allowing them to adhere to microtextures on the PDMS involved a multi-step process. First, PDMS stamps are made of varying thickness, then they were placed in a plasma cleaner and exposed to Argon for 1,3, and 5 minutes at 30 Watts, 8-10 MHz, and ~1000microTorr. Then, samples were exposed to N-isopropylacrylamide (NIPAM) via immersion into a polymer solution and via dropping that solution onto samples and baked at 3 hours or 5 hours. Cell detachment analysis, goniometer experimentation, and SEM images showed that a 1 minute Argon gas exposure, with 1 minute of NIPAM immersion and a 3 hour bake yielded the most successful layer that lifted cells without inhibiting the PDMS microtexture. Future work involves

optimizing the device to lift all cells exposed to the channel, as well as further corroborating its efficacy.

Acknowledgements

First and foremost, I'd like to thank Natalia Higuita-Castro for her seemingly inhuman amount of patience, as well as the whole of the Nanomedicine lab for their support. I'd like to thank my adviser, Daniel Gallego-Perez, for far exceeding the job description of "adviser." I'd like to thank Tanya Nocera for all the hours of advice, support, and wisdom; as well as Ben Jones for instruction and time in learning how to operate the equipment and machinery in Bevis.

Thank you to my Father for his support and dedication. Finally, thank you to Sam for her love, support, and access to her hulu account for those 3 hour bakes. Oh- I forgot one, thank you to my best friend, Fred Williamson, for his supervisory prowess.

Table of Contents

List of Figures

Introduction

Cellular migration is the process by which cells change position in response to a stimulus. This mechanism is vital in multicellular organisms and plays a significant role in immune responses, gastrulation and coagulation[1]. Cell migration is also the primary methodology behind the development of secondary tumors and further metastasis of many cancers[2]. Cancer metastasis via cellular migration can occur in a variety of ways, along many different physiologies. Canalicular spread is the metastasis along canalicular spaces such as the subarachnoid space, lymphatic spread is metastasis via regional lymph nodes, Hematogenous spread is most common for sarcomas and involves penetration into blood vessels to form secondary tumors in distant organs, and Transcoelomic spread is the movement of cells along body cavities[3-6]. Various primary tumors are also capable of metastasis via the release of a single cell, as opposed to the more commonly depicted cell cluster. One such cancer, Glioblastoma Multiforme (GBM), exhibits single-cell metastasis via high velocity along cranial microstructures[7].

GBM affects 2-3 adults for every 100,000 and accounts for 52% of all primary brain tumors. While not the most prevalent cancer, it remains among the deadliest. Of the 22,850 adults diagnosed in 2015, 15,320 died within the same year[8]. Glioma Stem Cells are highly invasive, and it is hypothesized that GBM's fatality is partially attributed to the stem cells' deleterious nature. The ability to isolate these cells for single cell and genetic analysis would promote therapeutic discovery to target phenotypically similar cells. Thus, there exists a need to sort cells based of unidirectional velocity and microtexture adherence. However, velocity-dependent cell separation techniques and devices are not very common because many cells display random motility on two-dimensional substrates[9].

Point-of-Care (POC) medical devices are a focal point of precision medicine and expanding healthcare to underserved populations. POC testing is the practice of performing medical testing outside of the clinical laboratory, oftentimes by professionals other than physicians, to a) increase healthcare availability to underserved populations b) decrease healthcare cost c) increase patient willingness to undergo diagnostic testing and d) take advantage of precision advances in the past 5-10 years (including the application of "big data" to medicine). POC testing can be broken down into four primary areas: self-monitoring (SM), and community testing in the emergency department, general practice, and pharmacy[10]. Common examples of POC testing include self-administered glucose reader chips, any of many "lab-on-a-chip" devices reaching market, and global health diagnostic devices for detection of infectious disease. Until recently, glucose sensors and lateral flow chips were the two primary POC devices at market. Technology miniaturization, specifically the development of handheld devices, as well as the development in complexity of technologies such as Raman spectroscopy and Polymerase Chain Reaction (PCR) have led to a dramatic increase in POC device research in recent years[11]. One such example is the Precise Advanced Technologies and Health Systems for Underserved Populations (PATHS-UP) Engineering Research Center National Science Foundation grant, dedicated towards the construction of POC devices for global healthcare.

POC style devices are also becoming more common, and are alike to POC devices in many ways. The primary difference is that POC style devices often require lab equipment or a highly controlled environment at a given point (not the entirety) in the methodology to function properly. POC style devices are also important, as they represent not only the growing trend of recognizing the importance of POC testing, but also can decrease healthcare costs. One common example of a POC style device are lab-on-a-chip to enumerate circulating tumor cells (CTC) in

the bloodstream. The device itself is POC, but often requires fluorescence microscopy to collect data and complete a diagnosis. The development of a POC proof-of-concept cell sorter would not only enable therapeutic discovery for cancer research at a high cost effectiveness, it could help burgeon POC cell sorting from its infancy.

Fluorescence activated cell-sorters (FACS) are a common method of cell-sorting, but require significant instrumental set up. This includes optics, computer hardware, a dynamic focusing apparatus (usually microfluidics driven), and more. Additionally, FACS methodologies can yield inconsistent cell survival rates for different cell lines which is limiting for post-assay analyses. While the data provided via FACS is substantial and can be used for therapeutic discovery, the expensive and prohibitive set-up will limit the functional range of a point-of-care cell sorter[12].

For cancer diagnostics and prognostics at the point of care, the aforementioned CTC enumeration devices are currently the most well-developed option. CTC enumeration devices vary in design, but across-the-board share similarities in mode of action. Most are adhesion driven, using anti-Epithelial Cell Adhesion Molecule (anti-EPCAM), surface area maximization, and flow manipulation to trap cancer cells drawn from the bloodstream into micropatterned edges or traps with fluorescent tags for further analysis. Unfortunately, this technique is also fatal for the cell and would limit further analysis[13-15].

The application of "smart polymers" to cell sorting is still in its infancy, as they are generally applied to drug delivery, protein folding, and tissue engineering[16]. However, the uniqueness and novelty of velocity-driven cell sorting is a strong application for smart polymers. Smart polymers are materials that can selectively actuate and functionalize based on the presence of a stimuli. Actuation capabilities range from swelling (fluid absorption) to physical

conformation and polarity changes. Stimuli, depending on the polymer, can exist as light, temperature, pH, current, magnetic field strength, and electric field strength. Depending on the stimuli, polymers can be actuated in microdomains or macrodomains. One such class of smart polymer of interest is the Lower Critical Standard Temperature (LCST) thermoresponsive polymer. Thermoresponsive polymers feature temperature driven actuation, and the most common response is conformation change. Thermoresponsive polymers have two primary subcategories: LCST and Upper Critical Standard Temperature (UCST). LCST thermoresponsive polymers exist in a hydrophobic state above a given temperature, and as a hydrophilic material below that given temperature. UCST thermoresponsive polymers exist as a hydrophilic material above a given temperature and as a hydrophobic material below said temperature. Due to its biocompatibility, the most common LCST thermoresponsive polymer is Poly(N-isopropylacrylamide) (PNIPAM), which exhibits a LCST of approximately 31 degrees Celsius that increases in effectiveness until approximately 20 degrees Celsius[17].

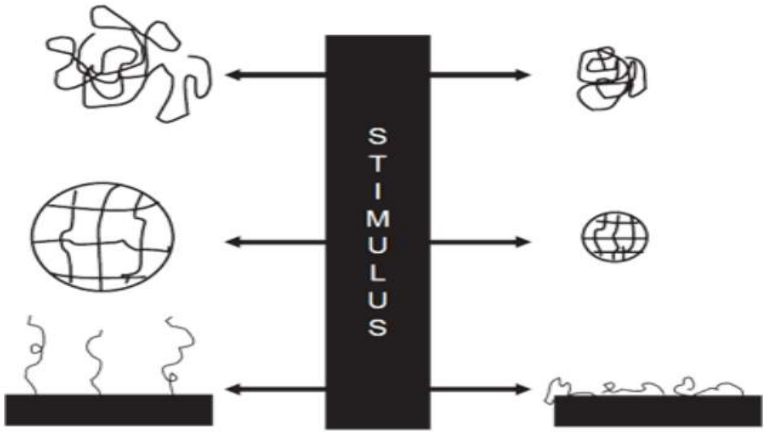

Figure 1. Actuation of Smart Polymer. *Smart polymers undergo a conformational change when exposed to a stimuli. This stimulus can take the form of light, heat, pH and more. In addition to stimulus variety, polymers adapt differently depending on their state. In descending order: linear free chains, gelatinous form, surface modification. Adapted from Sigma Aldrich*

In 2012, Rayatpisheh et al. utilized PNIPAM and plasma treatments to enable ultrathin layers of smooth muscle cells to be grown and detached in order to create muscle cell sheets[18].

Cells were seeded onto PNIPAM-coated Polydimethylsiloxane (PDMS), allowed to achieve confluency, and then removed via PNIPAM actuation over approximately 45 minutes. As the PNIPAM reached and surpassed its LCST, it changed conformation and released cells into the media in favor of binding to water in the cell culture media. While an effective approach for sheet tissue engineering in a lab setting, this methodology is not directly translatable to a POC-style velocity-dependent cell sorter as it actuates over an extended time period, features significant and expensive lab equipment for both device fabrication and actuation, and lacks precision. However, the method of PNIPAM actuation can be altered to feature the above characteristics[19].

Here, we present the fabrication and testing of a device capable of sorting cells based off velocity and adherence to microstructure. This device can be cheaply manufactured, and actuates in the point of care style- requiring minimal equipment and actuation time beyond a 24 hour incubation for cell growth and movement.

Materials and Methods

Microfluidics

The device constructed consists of multiple layers. First, a microfluidic channel 500 micrometers deep, 3.2mm in width, and 11mm in length is fabricated onto the bottom of a 6 well plate made of polystyrene via a knee milling. The inlet and outlet are accessible from the bottom of the device via Tygon tubing, syringe, and precision tip. The device is placed on an elevated stage during fluid flow to allow easy access. First prototypes featured a PDMS channel to be placed into each well-plate, but it was found that that creating the channel into the device itself allows for simpler actuation. Devices were actuated via syringe pump, 1mL/min flow rate for up to 10 minutes.

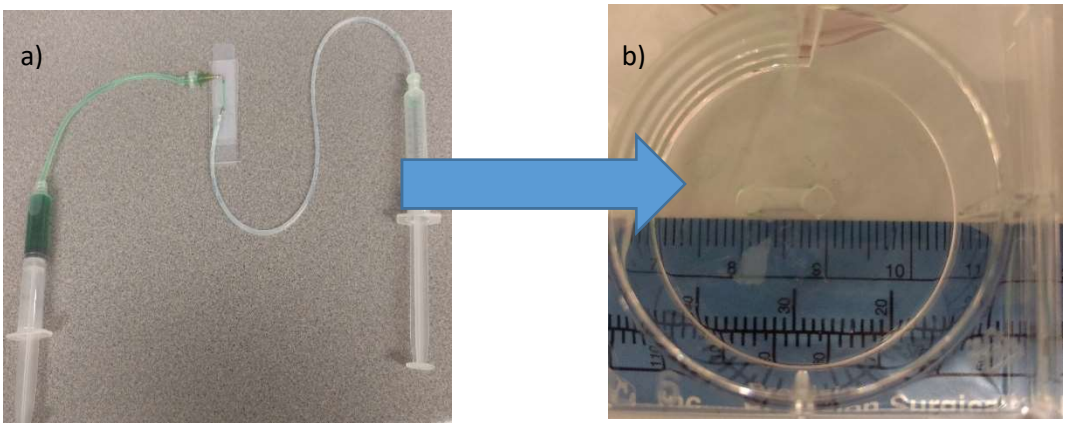

Figure 2: Microfluidic Prototype and Final Design. *a) Early prototype, featuring flow of dyed water through channel to be placed in well b) Final design, featuring 11mm channel milled directly into plate. Connections to each sample will be made at the syringe leur lock. Dyed fluid is drawn through the channel and up the barrel of the empty syringe*

In application, the channel can be placed at any location within the plate to accommodate for a given cell velocity.

PDMS Stamp

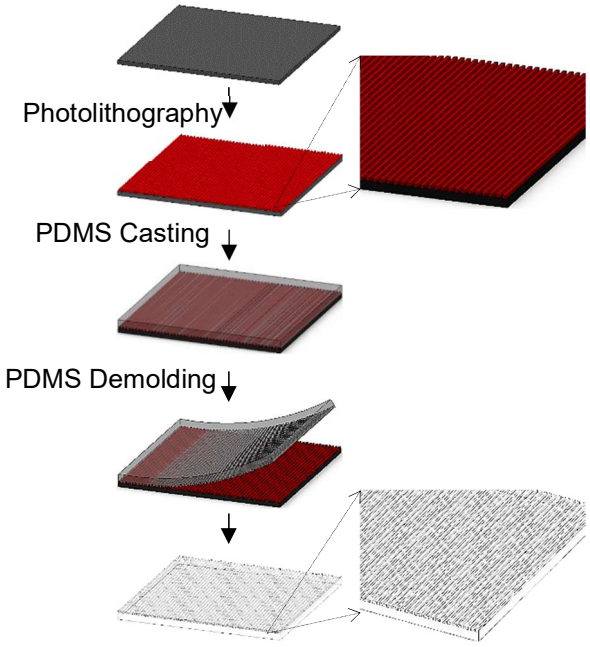

Figure 3: Platform Fabrication. *Adapted from Duarte-Sanmiguel et al.*

PDMS microtextured surfaces were fabricated from silicon masters. Masters were photolithographically patterned via standard UV photolithography with negative photoresist. Masters featured ridges 2µm wide and 1.5µm tall spaced 2µm.

Polydimethylsiloxane (PDMS) stamps were created from the Silicon Masters via replica molding. Sylgard 184 Silicone Elastomer Kit 10:1 Elastomer:Curing Agent was used. PDMS was mixed and desiccated pre-spin coating 3x for five minutes each. Next, PDMS was spin coated onto the wafer via Laurell WS-650MZ-23NPPB for one minute at 600RPMs to ensure minimal thickness. Next, samples were desiccated 3-5x for 10 minutes and cured at 60 ^{o}C for two hours or left to cure at air temperature for a minimum of 72 hours and allowed to rest. The wafer featured ridges two micrometers thick, separated by two micrometers.

PDMS was then cleaned to remove particulates, and sectioned into 15mm square samples.

PNIPAM Coating

Poly(N-isopropylacrylamide) (PNIPAM) was purchased as N-Isopropylacrylamide (NIPAM) from Sigma Aldrich (415324-10g 97%) and then placed into a 15%-wt solution. PDMS samples were treated at 30W, 8-10MHz and ~1000 microTorr for one, three, and five minutes with Argon Gas in a Harrick Plasma PDC-001 Expanded Plasma Cleaner. After treatment, samples rested for 15 minutes. They were then placed in NIPAM solution for either one or five minutes. Following immersion, baking occurred at 65°C for three or five hours to polymerize the NIPAM. After baking, samples were stowed for 48 hours.

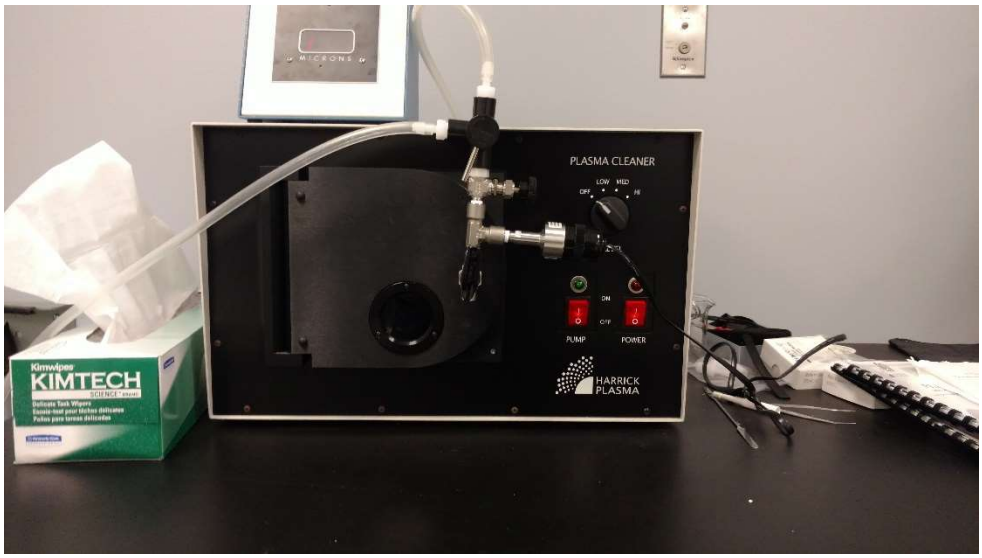

Figure 4: Harrick Plasma Plasma Cleaner.

Non-Precise Cell Lifting Assay- Cell Seeding

After 48 hours, samples were sterilized via Stratalinker UV Crosslinker. 200,000 A549 (adenocarcinoma alveolar cells- human) were seeded in Delbucco's Modified Eagle Media (10% Fetal Bovine Serum, 1% Anti-Anti) and allowed to grow for 24 hours.

Precise Cell Lifting Assay- Cell Seeding

After 48 hours, samples were sterilized via Stratalinker UV Crosslinker. 300,000 A549 (adenocarcinomic basal epithelial alveolar cells- human) were seeded in Delbucco's Modified Eagle Media (10% Fetal Bovine Serum, 1% Anti-Anti) and allowed to grow until at least 70% confluency had been achieved.

Non-Precise Cell Lifting

First, cell media is discarded and replaced to prevent dead cells from interfering with the assay. Then, .5mL 4 degree Celsius phosphate-buffered saline (PBS) is added to the well-plate and allowed to sit for five minutes. Media is then collected and cells counted via hemocytometer.

Precise Cell Lifting

Tape is placed on the bottom end of the device, covering the channel inlet and outlet. Using precision tips, tape is removed from the outlet and a small incision is made in the inlet. The tip is then inserted into the inlet and the syringe is loaded with cold fluid and affixed to a syringe pump. Fluid flows for 1mL/min for up to 10 minutes.

Channel Placement

The location of the channel can be altered as necessary to target cells of a given velocity. Aggressive cells would feature a higher velocity, and thus would move farther along the sample over a given period. Cells with a lower velocity would be best captured with channels nearer to the seeding site.

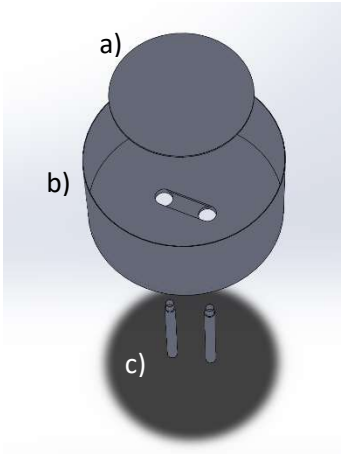

Figure 5: CAD Rendering of Final Design. *a) Treated PDMS stamp b) Milled 6 well plate c) Inlet and outlet precision tips. The diameter of a single well in a 6 well plate is approximately 35mm.*

Contact Angle Quantification

To ensure surface activation, samples were of each fabrication type were split into an activated ("COLD") and non-activated group ("HOT"). Activated group samples were sealed and placed on ice for up to ten minutes. Non-activated samples were placed on a hot plate set to ~40 degrees Celsius to mimic incubator conditions. Samples were placed on a goniometer such that a clear image of the surface was visible. Then, 8μL of water was placed on the sample, and an image of the water's shadow was immediately captured.

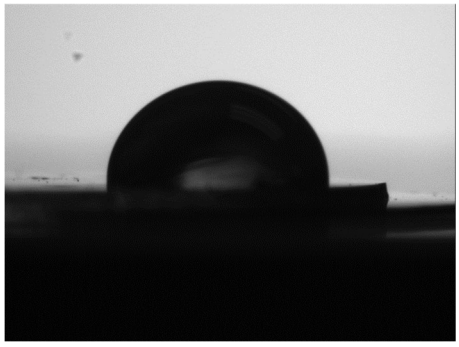

Figure 6: Goniometer Image*, 1 minute exposure to Argon plasma, 1 minute immersion in 15 wt. % NIPAM*

Results

Several assays were conducted to ensure efficacious lifting of cells. First, samples were fabricated with variable polymerization bake time (bake time), plasma cleaner exposure (exposure), and immersion time (IT). Samples were baked for either three or five hours at ~65 degrees Celsius; exposure occurred at either one minute, three minutes, or five minutes. Finally, samples were immersed in NIPAM solution for either one minute or five minutes. The first assay conducted was external contact angle determination of a drop of water to analyze activation of the polymer with varying temperature. Next, cells were seeded on the surface to ensure the PNIPAM layer did not interfere with cell alignment due to the microstructure. Then, SEM images of sample surfaces were analyzed. Cells were then lifted from the surface without precision and then with precision once the device was constructed. After samples underwent 48h rest following bake time, it was immediately seen that five hour bake samples featured visible quantities of PNIPAM. This was found to interfere with goniometer measurements, and was hypothesized to limit cellular adherence to PDMS topography. Thus, five hour bake samples are not included in any analysis.

Goniometer Measurements

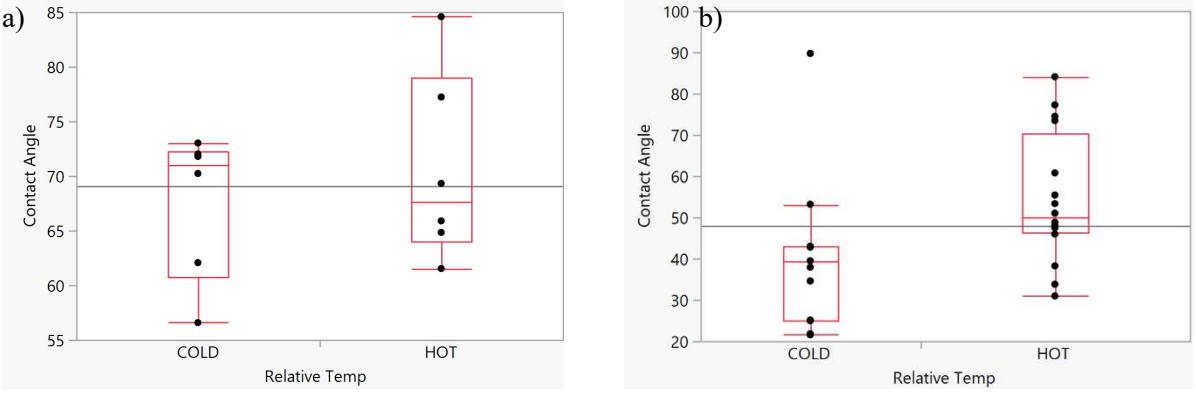

Figure 7: Contact Angle at two relative temperatures for flat PDMS *b) with and a) without PNIPAM. Addition of PNIPAM statistically significantly reduces contact angle for cold samples (p=.0157)*

As seen in figure 2, there was a statistically significant difference in measured contact angle between the actuated and non-actuated samples of PNIPAM coated PDMS. The control, figure 2a, demonstrated no such change over varying temperature. This indicates that the PNIPAM coating was able to functionalize and affect the hydrophilicity of the surface. The next step is to indicate which fabrication methodology best affects the surface hydrophilicity. Figure 3 displays the effect of exposure time on surface polarity.

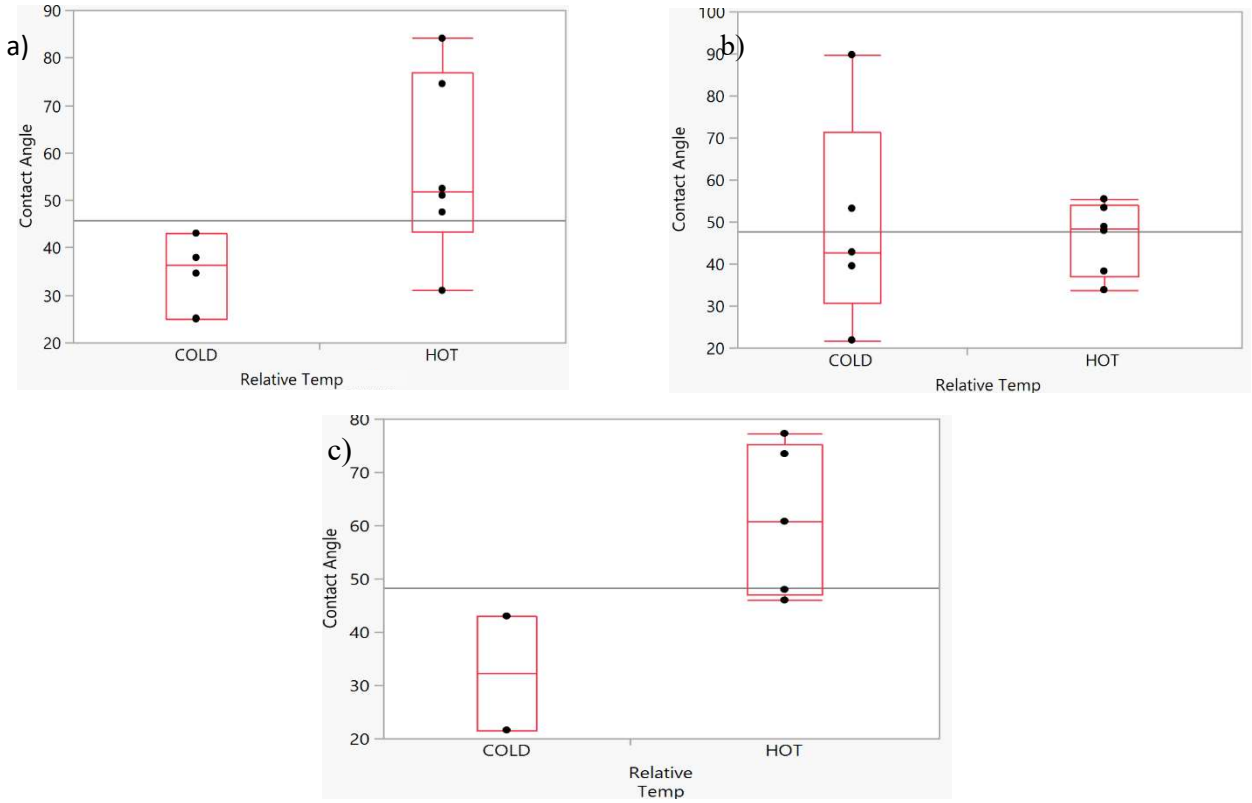

Figure 8: Contact Angle at two relative temperatures for flat PDMS with PNIPAM coating. *a) 1 minute exposure, b) 3 minute exposure, and c) 5 minute exposure. For 1 minute exposure: p=.0191, for 5 minute exposure: p=.0072.*

Both one minute and five minute exposure times statistically significantly affect surface polarity. For many samples, with increased frequency at five minutes exposure, the relatively cold water would maximize contact with the surface as it was placed. This cannot accurately be contributed to the polarizing effect PNIPAM had on the PDMS surface. It is possible that this was caused by PNIPAM polymer attracting the fluid via adhesive forces. Thus, those samples

were eliminated from analysis. As a result, the low sample size of n=2 for five minute exposure (COLD) requires more power to reach a conclusion. It is hypothesized that the addition of PNIPAM (regardless of actuation) may have a lesser effect of polarizing the surface. Thus, the averages observed for non-actuated samples are still lower than the values observed for PDMS without PNIPAM. This effect could have contributed to the lack of significance found at three minute exposure. After establishing that a one minute exposure time will best polarize the surface, it was necessary to find the ideal fabrication recipe to maximize PNIPAM actuation.

Figure 4 displays the comparison of each individual fabrication methodology at cold and hot conditions. Not pictured is 5 minute exposure, 5 minute immersion; as the aforementioned condition wherein fluid immediately maximizes surface contact made data collection difficult. This is also observed as n=2 for hot and cold 5 min exposure, 1 min immersion and 3 min exposure, 5 min immersion. The data show that 1 min exposure, 1 min immersion consistently provide the greatest level of actuation and surface polarization from non-actuated conditions. Ultimately, it is inconclusive whether longer exposure times (3 minutes & 5 minutes) can satisfactorily change surface polarity. However, for the scope of designing the cell sorter; goniometer measurement was satisfactory and 1 min exposure, 1 min immersion was found to statistically significantly alter PDMS surface properties as temperature changes.

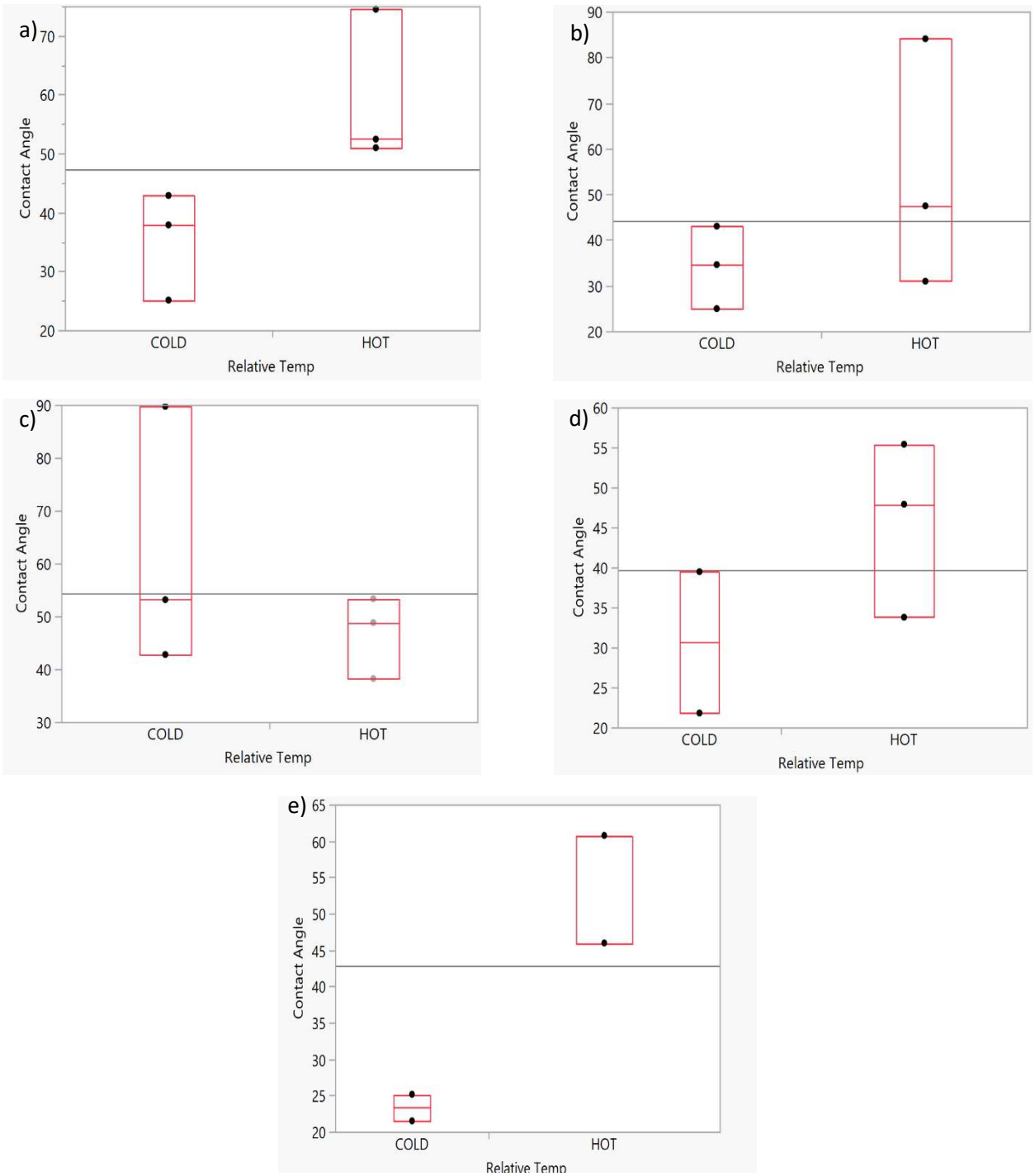

Figure 9: Contact Angle Differences for actuated and non-actuated Flat PDMS with PNIPAM coating *a) 1 min exposure, 1 min immersion: p=.0339 b) 1 min exposure, 5 min immersion c) 3 min exposure, 1 min immersion d) 3 min exposure, 5 min immersion e) 5 min exposure, 1 min immersion. 5 min exposure, 5 min immersion not pictured.*

Microtopography Adherence

While it is necessary that the surface be functionalized for polarity modification, it is equally important that seeded cells will still respond to patterned PDMS. To ensure that this is the case, A549 cells were seeded onto the functionalized surface and allowed to reach confluency. Images were then captured to perform a qualitative analysis of cellular alignment with topography. Figure 8 displays that a 1 min exposure, 1 min immersion treatment and functionalized surface did not interfere with cellular adherence to surface topography.

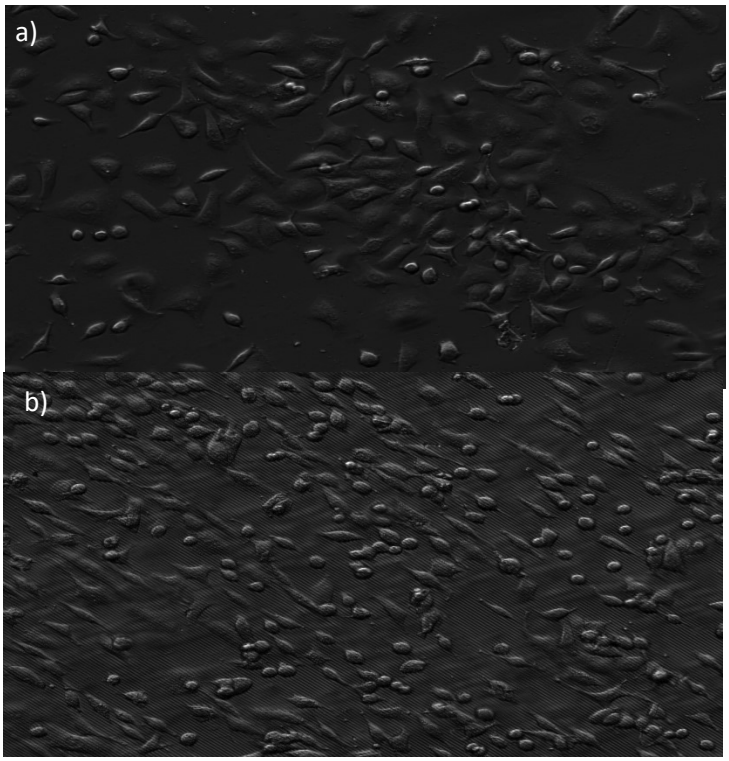

Figure 10: Cell Adherence to microtopography. *a) Control b) 1 min exposure, 1 min immersion*

Non-Precise Cell Lifting

Before the channel was constructed and tested, it needed to be confirmed that cells could be lifted through surface activation. According to the methodology described previously, cells were successfully lifted. Figure 9 displays this data. The "No PNIPAM" group are cells that

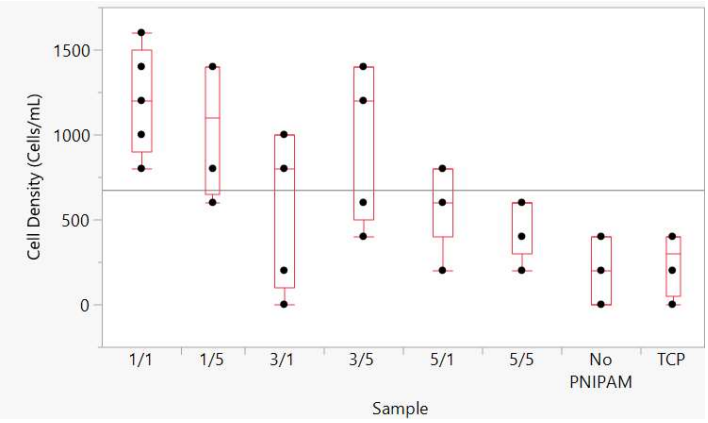

Figure 11: Figure 12: Cell Detachment as a Result of Addition of 4 °C .5mL PBS to 2mL DMEM 10% FBS 1% Anti-Anti. *A/B where A is exposure time in minutes and B is immersion time in Minutes*

were seeded directly onto PDMS with no PNIPAM functional layer. TCP group featured cells seeded directly onto Tissue Culture Plates. Significance was observed for 1/1 vs. No PNIPAM, p<.0001; 1/1 vs. TCP, p=.0002; 1/5 vs. No PNIPAM, p=.0006; 1/5 vs. TCP, p=.0019; 3/5 vs. No PNIPAM, p=.0006; and 3/5 vs. TCP, p=.00021. These results support the hypothesis that this methodology is capable of releasing cells into the media that were previously adhered to the surface. Approximately 175,000 cells were seeded per well in a 12 well plate 24 hours before this analysis was conducted. Without consideration of cell replication, only ~.6-1.0% of cells regularly detached from statistically significant samples. This low detachment rate could affect the efficacy of this device as an effective tool.

Precise Cell-Lifting

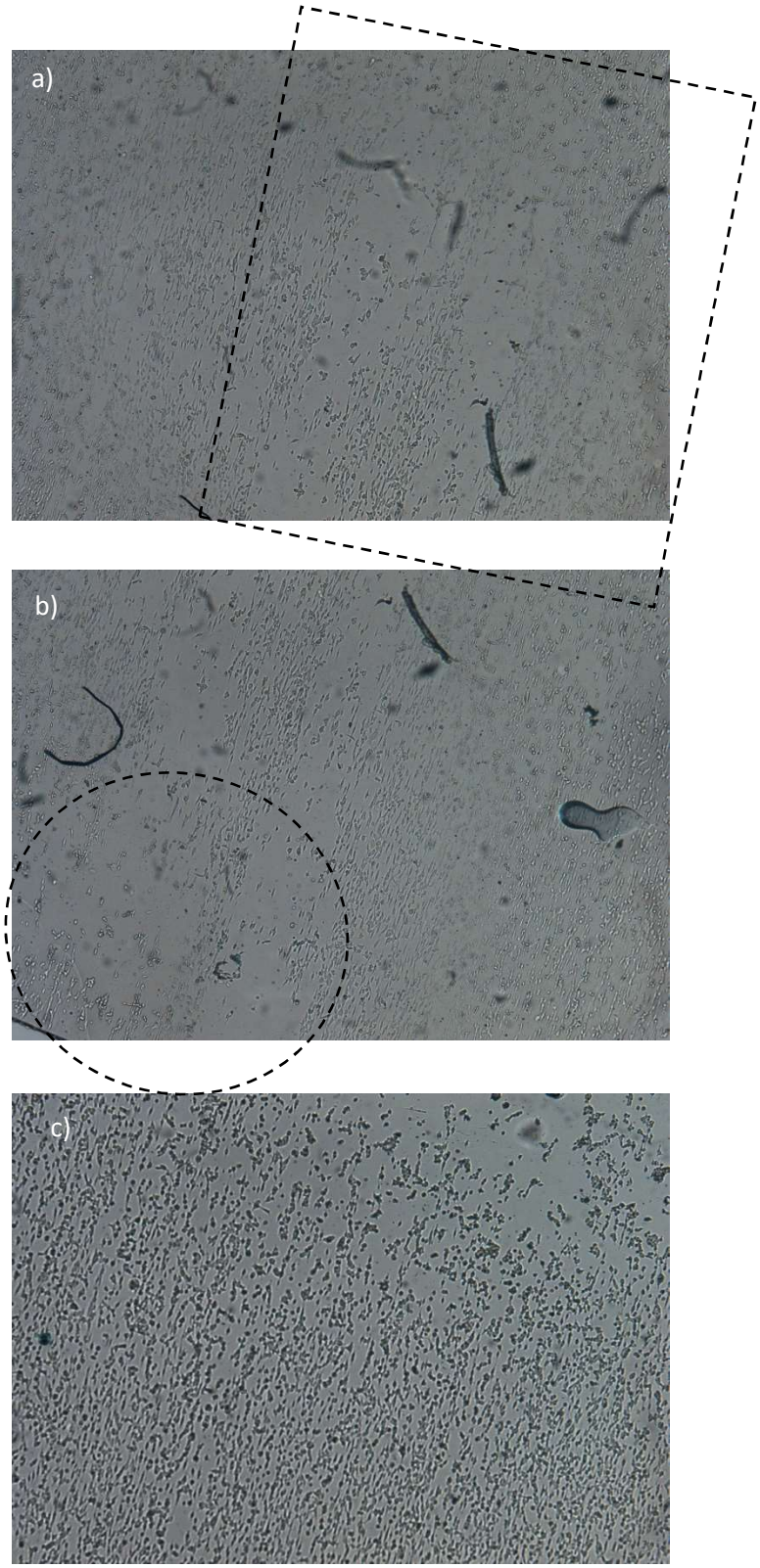

Figure 12: Precise Cell-Lifting, Sample 1. *a) lifting at estimated channel location b) lifting at estimated inlet location c) lack of lifting observed elsewhere on plate. All images 4x magnification*

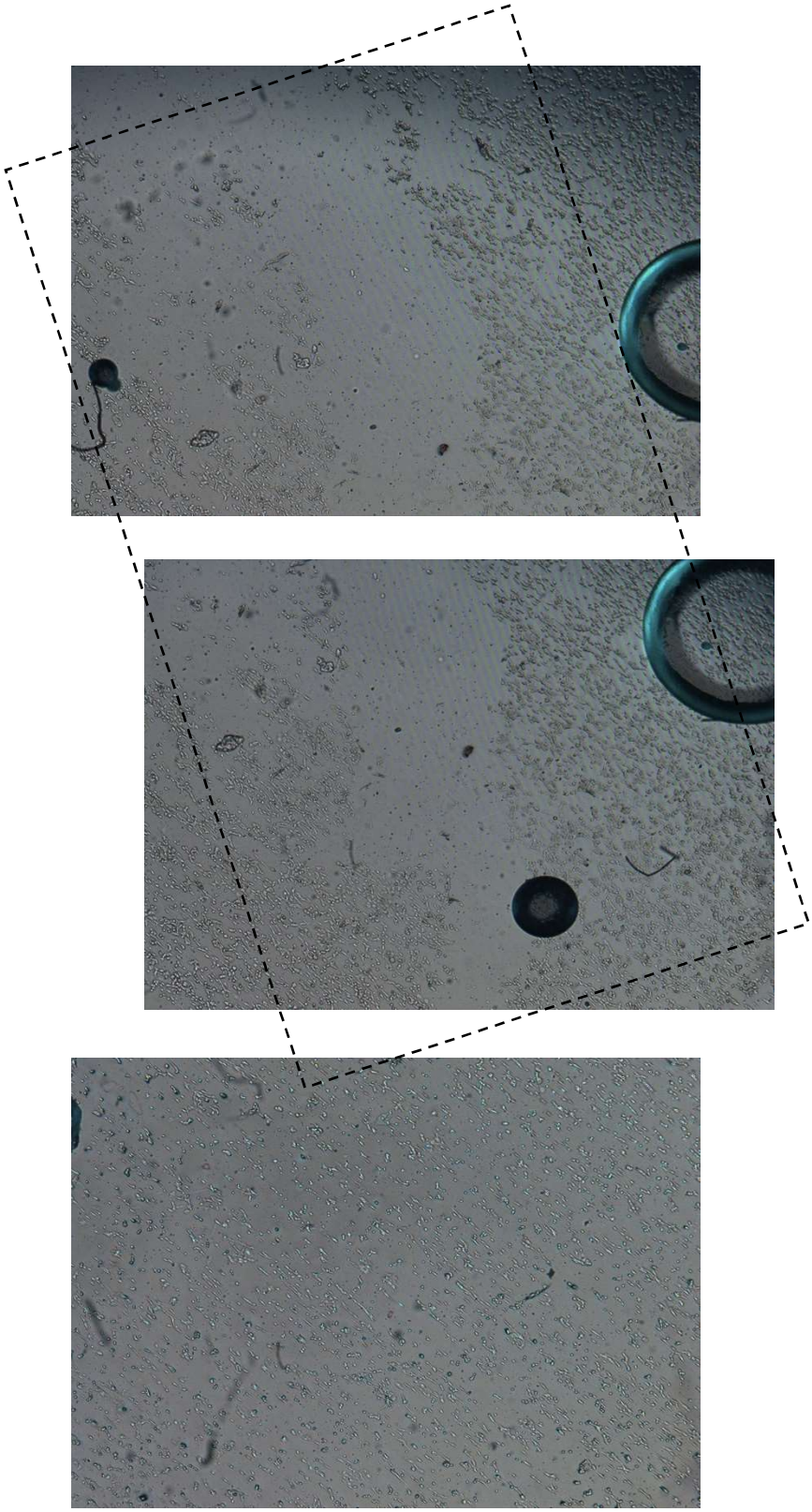

Figure 13: Precise Cell-Lifting, Sample 2. *a) lifting at estimated channel location b) lifting at estimated channel location (cont.) c) lack of lifting observed elsewhere on plate. All images 4x magnification*

As a proof of concept that cells could be selectively lifted with application of a microchannel within the completed device, the above data was collected. For a total of five samples examined, two completed testing. As indicated by the captured images, selective lifting occurred in space estimated to be directly above the channel and inlet/outlet. Figures 12 and 13 both feature a control, indicative of the level of confluence observed elsewhere on the plate. Each sample was scanned for duplication of the observed lifting, and in both samples, duplicate patterns of the same scale or larger were noticed.

Discussion & Conclusion

Here we present the complete fabrication and testing of a prototype device to be used to instigate therapeutic agent discovery for GBM. GBM metastasizes via single cells escaping the primary mass and following cranial microstructures to a new location, which make it difficult for modern chemotherapeutic agents and radiation to target and kill those cells. Isolation of those cells by taking note of their increased velocity and adherence to microstructures is a novel concept, as a need for velocity-dependent cell sorting has not existed beforehand. Through the application of microfluidics and thermoresponsive polymers, such a device was constructed and tested to function acceptably.

While the device has been proven to function well enough for a proof-of-concept, more work is needed to confirm and enhance its efficacy before it is used for therapeutic discovery. Firstly, the application of Energy Dispersive Spectroscopy (EDS) would support the claim of an even coating of PNIPAM on the surface. Next, scaling the channel down in size to work on the micro-level would be ideal to target and collect cells for single-cell clonal analyses. Additionally, more formal packaging- including a dedicated stand for the device and standardized placement of channels would make the device more consistent in its functionality and easier to work with as a research tool.

One issue that needs to be addressed is the translatability of the device across different users, and its consistency in action. In the fabrication process, it is easy to visually identify samples that will fail. This is done by waiting 48 hours after post-bake and discarding samples with visible PNIPAM, and is not a frequent occurance for the optimized recipe (1 min exposure/ 1 min immersion/ 3 hour bake). As it applies to testing- this was found to be a major fault. For the five test-samples prepared, only two completed the assay. While part of this can be

contributed to the need for practice and familiarity with the device, it is believed that this issue stems from the fragility of the a) seal between the PDMS and the plate and b) the thickness of the PDMS. By using a precision tip, it becomes incredibly easy to lift the PDMS or pierce it when performing the assay. This is especially the case when the PDMS is prepared at 600 RPM for 1 minute, resulting in an ultra-thin layer. A thin-enough tip can also cause backflow if the puncture of the tip through the tape is too large. Matching the inlet with a tip of proper size could rectify this issue, as it would prevent having to insert instruments into the channel to actuate flow. He

It was also found that the cells being lifted are not consistently located within the channel. While sample size is inadequate (n=2), for sample one it was found that that the greatest lifting occurred in no specific region of the channel cross section. However, for sample 2 it was found that lifting occurred explicitly in the center of the channel. Further experimentation is necessary to validate/invalidate this claim.

As a point-of-care style device, this proof-of-concept was successful. The cost of fabrication for a 6 well plate (not including cost of labor) is approximately $10. $7.20 of that cost is due to polymer purchasing. Device testing requires no special equipment and can be performed bench side. Fabrication equipment required are knee mill, oven, plasma cleaner, and spin coater. Rudimentary plasma cleaners and spin-coaters can be found in most microfabrication laboratories, and low cost-equipment was used in this work to that end. Fabrication (from polymer construction to cell seeding) takes 48 hours, and sample growth (from seeding to testing) varies depending on the cell type. However, only 5 hours of active labor is required to construct a batch of samples; which scales up quickly without added labor time. The biggest limitation to scalability is oven space.

The next iteration of this device could apply the principle of chemotaxis, using chemical stimuli to force cells in a specific direction. This would limit the effect of 2-dimensional random walk seen in cell movement over time. Additionally, the device could be actuated in a transwell plate, bypassing the need for microchannels and instead using double-sided textured PDMS to actuate lifting.

References

1. Mak, M.; Spill, F.; Roger, K.; Zaman, M. "Single-Cell Migration in Complex Microenvironments: Mechanics and Signaling Dynamics". Journal of biomechanical engineering. 138: 021004. doi:10.1115/1.4032188.
2. Zijl, Franziska Van, et al. “Initial Steps of Metastasis: Cell Invasion and Endothelial Transmigration.” Mutation Research/Reviews in Mutation Research, vol. 728, no. 1-2, 2011, pp. 23–34., doi:10.1016/j.mrrev.2011.05.002.
3. Chermet, Jacques, and Jean-Michel Bigot. “Inferior Vena Cavography in Hepatic and Intraperitoneal Diseases.” Venography of the Inferior Vena Cava and Its Branches, 22 June 1980, pp. 125–137., doi:10.1007/978-3-642-67579-9_8.
4. Carr, Ian. “Lymphatic Metastasis.” Cancer And Metastasis Review, vol. 2, no. 3, 1983, pp. 307–317., doi:10.1007/bf00048483.
5. Wong, Sunny Y., and Richard O. Hynes. “Lymphatic or Hematogenous Dissemination: How Does a Metastatic Tumor Cell Decide?” Cell Cycle, vol. 5, no. 8, 2006, pp. 812–817., doi:10.4161/cc.5.8.2646.
6. Tan, David Sp, et al. “Mechanisms of Transcoelomic Metastasis in Ovarian Cancer.” The Lancet Oncology, vol. 7, no. 11, 2006, pp. 925–934., doi:10.1016/s1470-2045(06)70939-1.
7. Gallego-Perez, Daniel, et al. “On-Chip Clonal Analysis of Glioma-Stem-Cell Motility and Therapy Resistance.” Nano Letters, vol. 16, no. 9, Oct. 2016, pp. 5326–5332., doi:10.1021/acs.nanolett.6b00902.
8. “Glioblastoma Multiforme.” AANS, www.aans.org/Patients/Neurosurgical-Conditions-and-Treatments/Glioblastoma-Multiforme.

9. Camley, Brian A., and Wouter-Jan Rappel. "Velocity Alignment Leads to High Persistence in Confined Cells." Physical Review E 89.6 (2014): n. pag. Web.
10. Price, Christopher P. “Evidence to Support Policy Decisions on Point-of-Care Testing.” PsycEXTRA Dataset, doi:10.1037/e519442012-011.
11. O’Kane, Maurice J. “Point of Care Testing—Current and Emerging Quality Perspectives.” Point of Care: The Journal of Near-Patient Testing & Technology, vol. 13, no. 1, 2014, pp. 1–5., doi:10.1097/poc.10.1097/poc.0000000000000008.
12. Cho, Sung Hwan, et al. “Human Mammalian Cell Sorting Using a Highly Integrated Micro-Fabricated Fluorescence-Activated Cell Sorter (MFACS).” Lab on a Chip, vol. 10, no. 12, 2010, p. 1567., doi:10.1039/c000136h.
13. Burt, B., T. Tilleman, R. Bueno, and D. Sugarbaker. "Use of Circulating and Tumor-infiltrating Myelomonocytic Cells to Predict Survival in Human Malignant Pleural Mesothelioma (MPM)." Journal of Clinical Oncology 28.15_suppl (2010): 10578. Web.
14. Sequist, Lecia V., Sunitha Nagrath, Mehmet Toner, Daniel A. Haber, and Thomas J. Lynch. "The CTC-Chip: An Exciting New Tool to Detect Circulating Tumor Cells in Lung Cancer Patients." Journal of Thoracic Oncology 4.3 (2009): 281-83. Web.
15. Murlidhar, Vasudha, Mina Zeinali, Svetlana Grabauskiene, Mostafa Ghannad-Rezaie, Max S. Wicha, Diane M. Simeone, Nithya Ramnath, Rishindra M. Reddy, and Sunitha Nagrath. "A Radial Flow Microfluidic Device for Ultra-High-Throughput Affinity-Based Isolation of Circulating Tumor Cells." Small 10.23 (2014): 4895-904. Web.
16. Grabauskiene, Mostafa Ghannad-Rezaie, Max S. Wicha, Diane M. Simeone, Nithya Ramnath, Rishindra M. Reddy, and Sunitha Nagrath. "A Radial Flow Microfluidic

Device for Ultra-High-Throughput Affinity-Based Isolation of Circulating Tumor Cells." Small 10.23 (2014): 4895-904. Web.

17. Roy, Ipsita et al. “Smart Polymeric Materials: Emerging Biochemical Applications.” Chemistry & Biology, vol, 10, no. 12, 2003, pp. 1161-1171, doi:10.1016/j.chembiol.2003.12.004
18. Rayatpisheh, Shahrzad, et al. “Argon-Plasma-Induced Ultrathin Thermal Grafting of Thermoresponsive PNIPAm Coating for Contractile Patterned Human SMC Sheet Engineering.” Macromolecular Bioscience, vol. 12, no. 7, 2012, pp. 937–945., doi:10.1002/mabi.201100477.
19. Schild, H. G. Poly(N-isopropylacrylamide): Experiment, Theory and Application. Prog. Polym. Sci. 1992, 17, 163−249